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The effect of arginine vasotocin on courtship behaviour in a blenniid fish with alternative reproductive tactics

L.A. Carneiro[1], R.F. Oliveira[1,*], A.V.M. Canário[2] and M.S. Grober[3]
[1]*Instituto Superior de Psicologia Aplicada, Rua Jardim do Tabaco 34, 1149-041 Lisboa, Portugal;* [2]*Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, 8000 Faro, Portugal;* [3]*Department of Biology, Georgia State University, MSC 8L0389, 33 Gilmer St. SE Unit 8, Atlanta GA 30303-3088, USA;* **Author for correspondence (Phone: 351 21 8811700; Fax: 351 21 8860954; E-mail: ruiol@ispa.pt)*

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Abstract

In a sex-role reversed population of the peacock blenny, *Salaria pavo*, two alternative male types are present: (a) older and larger nest-holder males that defend nests in which females come to spawn, and (b) younger and smaller sneaker males that mimic female-courtship behaviour and nuptial colouration in order to try to sneak fertilizations during spawning episodes. In this study the effects of exogenous administration of arginine vasotocin (AVT, 3 μg/g body weight) on the behaviour of nest-holder males, sneakers and females was tested. AVT induced the expression of female courtship behaviour in sneakers and female nuptial colouration in sneakers and in females, but failed to promote the expression of male courtship behaviour in both male types.

Introduction

Arginine-vasotocin (AVT) is a neuropeptide mplicated in the regulation of the expression of courtship behaviour in a variety of non-mammalian vertebrates (Moore 1992). In species with alternative reproductive tactics (ART), in which bourgeois (courting) males and parasitic (non-courting) males occur, there is an association between reproductive phenotype and soma size, number and/or mRNA expression of AVT neurons in the preoptic area (Foran and Bass 1999; Godwin et al. 2000; Grober et al. 2002).

Although the functional significance of these differences between alternative sexual morphs is not well established it is clearly associated with the expression of 'morph' typical behaviour (Foran and Bass 1999). However, these differences can be attributed either to the differences in the expression of alternative morphotypes or to the differences in the expression of courtship behaviour between morphotypes.

The peacock blenny, *Salaria pavo*, is an intertidal blenniid species, in which males defend a nest in a crevice and only the male provides parental care. In this species females also play an active role in courtship and thus both male and female courtship behaviours are present. Smaller and younger males engage in sneaking and adopt female-like behaviours and nuptial colouration, a behaviour that facilitates approaching and entering the nests during a spawning episode (Gonçalves et al. 1996). In S. pavo the expression of the bourgeois tactic is decoupled from the expression of courtship behaviour, due to sex-role reversal in courtship, which allowed to study the AVT correlates of ART vs. courtship.

The present paper investigated the effect of AVT on courtship behaviour in the three sex types of *S. pavo*: females, nest-holder males and sneakers.

Materials and methods

Individuals were captured at Culatra Island (Ria Formosa Natural Park, Portugal) and transported to the laboratory (Instituto Superior de Psicologia Aplicada, Lisbon) where they were kept in a community tank (120 × 50 × 40 cm) at room temperature (water tem-

Table 1. Effects of AVT intraperitoneal injections on sexual behaviour in nest-holder males, sneakers and females of *S. pavo*

	AVT	Control	Mann-Whitney U test (Z_{adj}, P value)
Experiment 1: Sneaker behaviour towards females			
Male nuptial colouration (s)	923.28 ± 97.94 (N = 14)	731.29 ± 116.01 (N = 15)	$Z = -1.96$ $P = 0.05$
Male courtship behaviour (acts/min)	0.29 ± 0.19 (N = 14)	0 ± 0 (N = 15)	$Z = -1.49$ N.S.
Experiment 2: Sneaker behaviour towards nest-holder males			
Female nuptial colouration (s)	886.49 ± 116.59 (N = 14)	434.47 ± 122.82 (N = 15)	$Z = -2.53$ $P < 0.05$
Female courtship behaviour (acts/min)	3.50 ± 0.61 (N = 14	2.53 ± 1.17 (N = 15)	$Z = -1.97$ $P < 0.05$
Experiment 3: Female behaviour towards nest-holder males			
Female nuptial colouration (s)	1031.56 ± 66.21 (N = 12)	533.16 ± 101.90 (N = 15)	$Z = -3.37$ $P < 0.001$
Female courtship behaviour (acts/min)	10.92 ± 2.16 (N = 12)	6.67 ± 1.74 (N = 15)	$Z = -1.64$ $P = 0.09$

perature = 20 ± 2 °C) and natural photoperiod (14L: 10D). Individuals were treated with intraperitoneal injections of either AVT (Sigma, 3 μg/g body weight) or with isotonic Ringer solution only (control) and their effects on courtship behaviour were investigated. Female courtship behaviour was defined as approaching a nest-holding male beating the pectoral fins and opening and closing the mouth while displaying female nuptial coloration (Patzner et al. 1986). Male courtship behaviour was registered when males performed figure 8 swimming and/or lead females to the nest entrance, or when they exhibited quivering behaviour while at the nest entrance. Usually these behaviours were performed while displaying a typical male nuptial coloration (Patzner et al. 1986). The courtship tests were intended to assess the effects of AVT on the expression of male courtship behaviour towards females (in nest-holder and in sneaker males) and on the expression of female courtship behaviour towards nest-holder males (in females and in sneakers). In the male courtship test, an ovulated female was introduced in the subject's tank and the behaviour of the individual towards the female was recorded. In the female courtship test the subjects were transferred into a tank ($70 \times 30 \times 40$ cm) containing an established nest-holder male (in a nest with eggs) and the female-like courtship behaviour of the sneaker towards the nest-holder male was recorded. The behavioural tests were videotaped with time code and subsequently analysed using video analysis (Observer v.5.0, Noldus Information Technology, Wageningen, The Netherlands). Due to the small size of the samples two-tailed non-parametric statistics were used with a alpha value et at 5%. All statistical procedures were performed using the software Statistica for Windows v. 5.0 (Statsoft Inc., Tulsa, OK, U.S.A.).

Results

In sneakers the AVT treatment increased the time spent in female nuptial colouration and the frequency of the female-like courtship behaviour displayed towards nest-holder males but failed to induce the expression of male courtship behaviour towards females (Table 1). Accordingly, AVT induced the expression of both nuptial colouration and courtship behaviour in females (Table 1), but failed to promote any expression of male courtship behaviour or nuptial colouration in nest-holders.

Discussion

The results of this study suggest that female courtship behaviour in both females and sneaker males is mediated by AVT but that male courtship behaviour is independent of AVT. These results are in agreement with the fact that in *S. pavo* the expression of AVT mRNA on a per cell basis is correlated with mating behaviour, rather than sex morphotype, and thus AVT mRNA expression in females and sneakers is associated with the production of courtship behaviour, whereas the number and size of AVT-ir cells in both male types is higher/ larger than in females, and thus is associated with sex morphotype, rather than with mating behaviour (Grober et al. 2002). Interestingly treatment of sneakers with 11-ketotestosterone (KT) silastic implants fails to induce any changes in forebrain AVT-ir cells, either number or size of cells, while it inhibited the expression of female-like courtship behaviour in sneakers (Oliveira et al. 2001a). Since nest-holders have higher androgen levels than sneakers (Oliveira et al. 2001b) it is predicted that the effect of KT on female courtship may be mediated by a reduction in AVT expression. Unfortunately, AVT *in situ* hybridisation assays were not performed in the above mentioned experiment (i.e. Oliveira et al. 2001a), and thus this hypothesis remains to be tested.

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